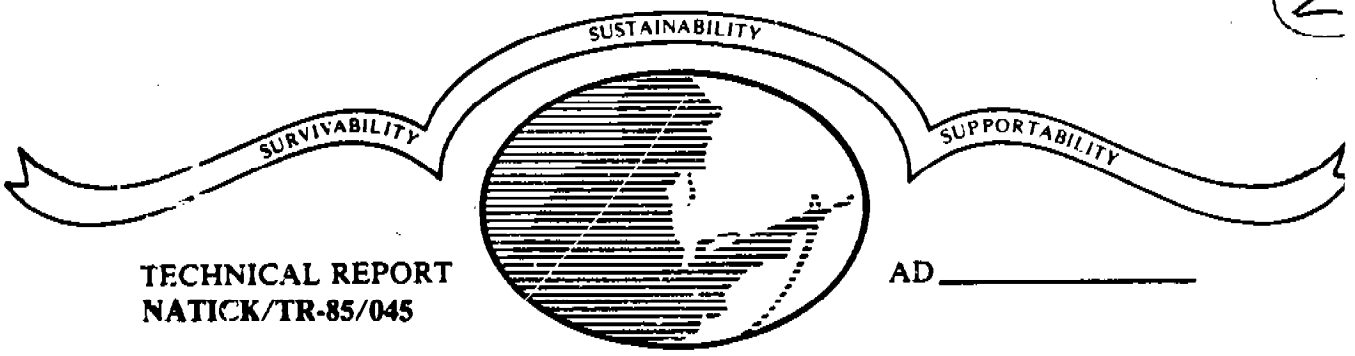

TECHNICAL REPORT
NATICK/TR-85/045

AD_____

BIOCONVERSION OF NITRAMINE PROPELLANT WASTEWATERS - TRIAMINOGUANIDINE NITRATE

BY
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AND
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JANUARY 1985

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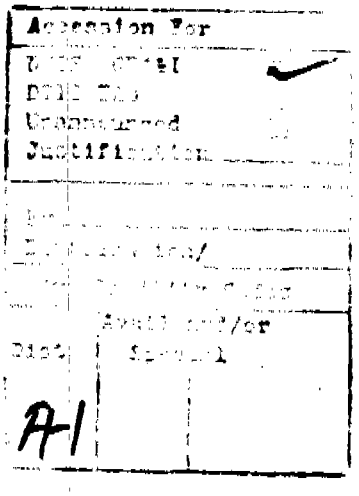

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TRIAMINOGUANIDINE NITRATE	ENVIRONMENTS
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POLLUTION ABATEMENT	TOXICITY

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The biodegradability of triaminoguanidine nitrate was determined under a variety of environmental conditions. Supplemental carbon was required in order for biodegradation to occur under aerobic or anaerobic conditions. Although triaminoguanidine nitrate is biodegraded under both aerobic and anerobic conditions, the nitrate portion of the salt will only degrade anaerobically (denitrification). The alternate carbon is required for this process as well.

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Triaminoguandine nitrate was found to be unstable under alkaline conditions resulting in the formation of carbohydrazide (diaminourea). No urea, guanidine cyanamide, cyanoguanidine or hydrazine were detected during the degradation of triaminoguanidine nitrate.

Resorcinol was also shown to be biodegraded, as has been previously reported in the literature. Isodecylpelargonate and Paraplex G-54 were only sparingly soluble in water.

Toxicity data collected from previously published literature are also presented.

PREFACE

Radford Army Ammunition Plant (RAAP) is establishing manufacturing capabilities for the production of low vulnerability (LOVA) propellant. In support of this effort, the pollution abatement requirements for nitramines at RAAP must be determined.

This effort was funded by the US Army Toxic and Hazardous Materials Agency (USATHAMA) under work unit P112.03.06 (W-76), 3?214166000 and 43214166000.

We wish to thank Dr. John H. Cornell of the Science and Advanced Technology Laboratory (SATL), Natick R&D Center, for his helpful discussions, Carmine DiPietro (SATL, Natick R&D Center) for his mass spectrometer analyses, and Mark Sharkey, Eric Myerson and Nancy Trumble (SATL, Natick R&D Center) for their technical assistance. We also wish to thank Norville Stanley, Allegheny Ballistics Laboratory, Hercules, Inc., PO Box 210, Cumberland, MD for supplying the triaminoguanidine nitrate.

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INTRODUCTION

Background

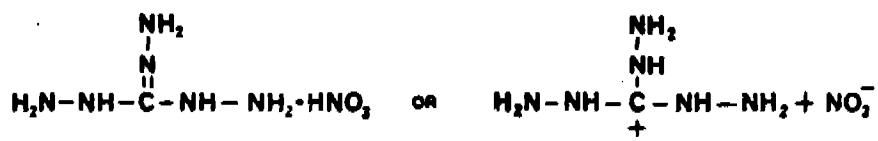
Nitrate propellant wastewaters are produced during the manufacture of various propellants, including RDX, HMX and triaminoguanidine nitrate (TAGN). Radford Army Ammunition Plant (RAAP) Radford, VA is establishing a production facility for low vulnerability (LOVA) propellant, which is expected to produce approximately 5.4 million lb (1.16 million kg) of LOVA propellant and 6.0 million lb (2.72 million kg) of ground nitramine per year.[1]

Wastewater from RAAP propellant manufacturing facility currently undergoes secondary treatment prior to discharge into the New River. The wastewater is combined with pre-treated wastewater from the nitroglycerine and nitrate ester manufacturing process into a central biological wastewater treatment operation. This facility contains a 1.25 million gal (4.73 million L) equalization basin and 12 rotating biological contactors operating in aerobic and anerobic modes.

In support of this facility, the pollution abatement requirements for nitramines at RAAP must be determined, including the potential for biological treatment of wastewater components. The biodegradability of RDX and HMX have been addressed in previous studies.[2-4] The potential for biological treatment of TAGN, and other wastewater components (resorcinol, isodecyl pelargonate, Paraplex G-54) will be addressed in this report.

Triaminoguanidine Nitrate (TAGN)

TAGN is a white crystalline powder with a molecular weight of 167.14 and the following structure:



The use of TAGN in propellants is advantageous for the following reasons: (1) TAGN contains a high percentage of nitrogen in hydrazine-type bonds which are thermodynamically favorable for propulsion (i.e., ammonium nitrate, guanidinium nitrate and triaminoguanidinium nitrate contain 35.0%, 45.9% and 58.7% nitrogen, respectively); (2) salts of triaminoguanidine have good thermal stability, (3) TAGN is compatible with other propellant or explosive ingredients, and (4) propellants based on TAGN produce low flame temperature

and low gas molecular weights while providing increased muzzle velocity and impetus, thus reducing life-cycle costs for the weapons.[5-7]

Limited information is available in the literature regarding the biodegradability of TAGN.[8]

At least three synthetic routes for TAGN have been published, from cyanamide, from guanidine nitrate, and from aminoguanidine.[7,9,10]

The toxicity data available for TAGN indicate that there may be reason for concern about this compound, although the conclusions of the individual reports were somewhat contradictory.

TAGN at concentrations up to 400 mg/L was found relatively nontoxic to Drosophila melanogaster.[11] Concentrations $\geq$ 2000 mg/L were toxic to adult Drosophila while at 1000 mg/L pupae and larvae production ceased and approximately half the adults were killed. Concentrations $\geq$ 250 mg/L were required to affect the reproductive potential of Drosophila. The authors concluded that TAGN has a relatively low toxicity and does not present a significant environmental or handling problem.

TAGN produced positive results in the Ames Salmonella mutagenicity test, the mouse lymphoma cell assay, and the unscheduled DNA synthesis assay, but negative results in the dominant lethal assay in rats and mice.[12] The authors concluded that TAGN had a high probability of being carcinogenic.

Davis et al.[13] reported an acute interperitoneal LD_{50} in mouse of 3.7 g/kg and TAGN caused bradycardia in the dog at intravenous levels above 50 mg/kg. These authors concluded that acute exposure to TAGN represents a relatively low hazard.

Teratogenic effects of TAGN were studied with pregnant rats treated with up to 8000 mg of TAGN per kg.[14] At the higher doses, maternal weight loss and litter reabsorption occurred. No increase in malformations was observed, although at the lower doses there was increased runting and perinatal death.

Resorcinol

Resorcinol is used as a stabilizer in the TAGN-propellant mixture. Resorcinol has been shown to be biodegradable in the published literature.[15,16] These reports have demonstrated that the aromatic ring undergoes ring scission with subsequent metabolism of organic fragments. The ring cleavage is accomplished through the incorporation of oxygen at the ortho position to the aromatic hydroxyl via monooxygenase enzyme activity. Dioxygenase enzymes are responsible for the subsequent ring scission. At least three metabolic pathways have been identified, as illustrated in Fig. 1.

Resorcinol has been found nontoxic and not carcinogenic in most studies reported in the literature.[17-19]

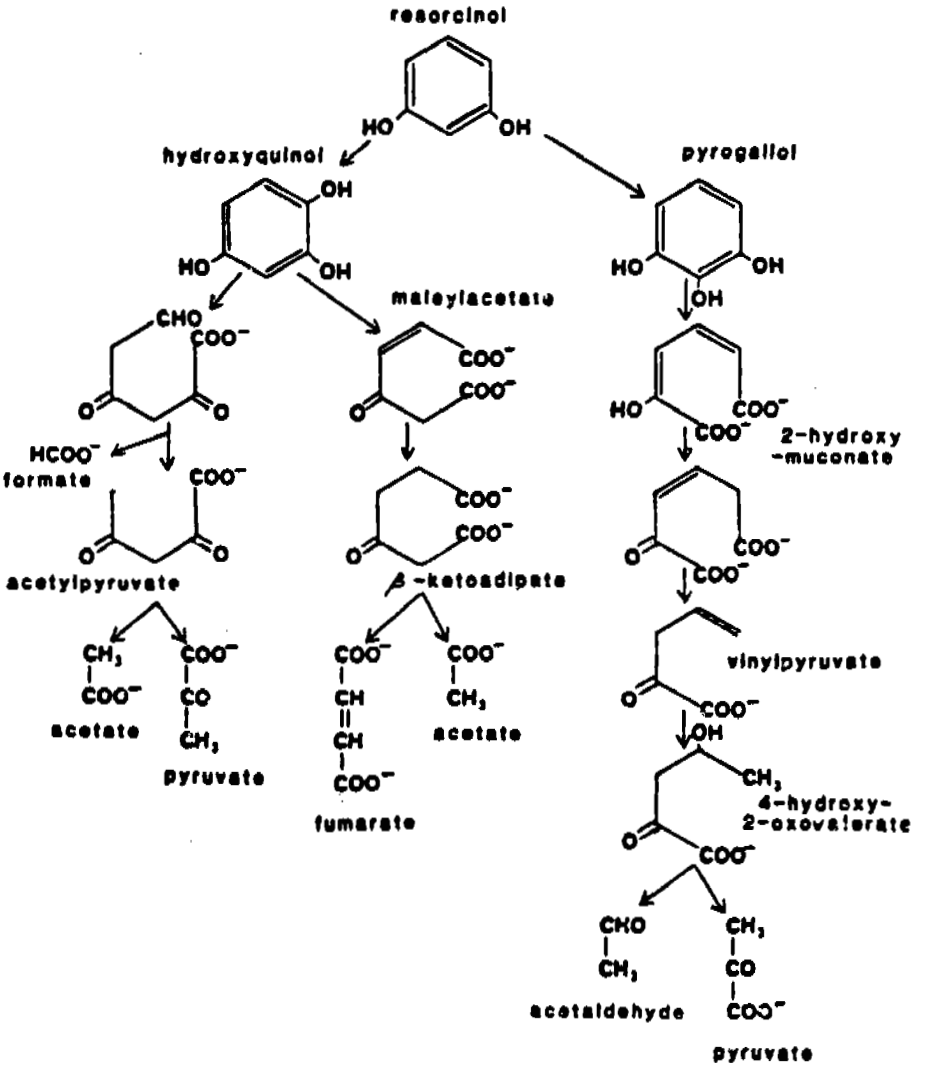
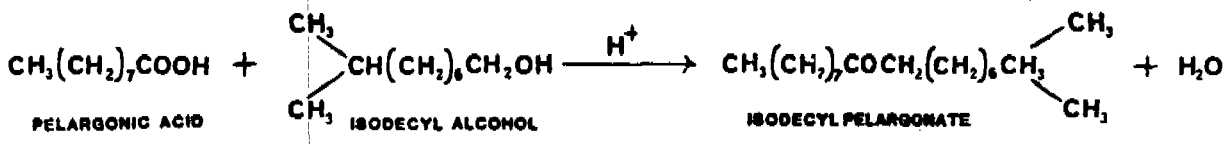


Figure 1. Metabolism of resorcinol as reported in the literature.

Isodecyl Pelargonate

Isodecyl pelargonate is a fatty acid ester of pelargonic acid (nonanoic acid) and isodecyl alcohol, which is used as a plasticizer in the propellant mixture:



Isodecyl pelargonate has negligible solubility in water and is relatively stable. There are no toxicological data available in the literature; however, it is presumed to be nontoxic.

Microbial and mammalian esterases, which hydrolyze esters of fatty acids, are common.[20-23] Andreev and Temnov[20] demonstrated the hydrolysis of methyl esters of pelargonic acid. The products of the esterase activity, acid and alcohol, are then subject to biodegradation; fatty acids undergo metabolism via B-oxidation.

Paraplex G-54

Paraplex G-54 is a mixture of aliphatic polyesters encompassing a range of molecular weights, which is used as a deterrent coating in the propellant mixture. Due to proprietary considerations, no further information was provided by the manufacturer regarding composition of this component.

Objective

The objective of this work was to assess the biodegradability of TAGN, resorcinol, isodecyl pelargonate and Paraplex G-54 in order to assess the potential for biological treatment of wastewaters containing these compounds.

MATERIALS AND METHODS

Chemicals

TAGN was provided by Norville Stanley, Allegany Ballistics Laboratory, Hercules, Inc., Cumberland, MD and recrystallized from hot distilled deionized water. Isodecyl pelargonate and Paraplex G-54 were provided by Hercules, Inc., Aerospace Division, RAAP, Radford, VA. Resorcinol, reagent grade, was purchased from Fisher Scientific Co.

UV/VIS Spectrophotometry

Ultraviolet and visible spectra of TAGN and microbially produced intermediates were obtained with a Perkin Elmer (Norwalk, CT) Lambda 3 UV/VIS spectrophotometer.

High Performance Liquid Chromatography (HPLC)

Resorcinol, melamine, nitrates, and nitrites were analyzed by HPLC with a Waters Associates (Milford, MA) system equipped with two M6000A solvent delivery pumps, a M730 data module, a M721 system controller, and a M710B WISP autosampler.

Resorcinol was determined at 254 nm on a C-18 reverse phase 10 um radial pack cartridge (Waters Associates). The mobile phase was 10% methanol in water (volume/volume) flowing at 2 mL per minute, the run time was 8 minutes, the injection volume was 25 uL and the retention time was approximately 5 minutes.

Melamine was analyzed at 229 nm on the same column as described for resorcinol, but with a mobile phase consisting of 50% methanol in water (volume/volume). The run time was 10 minutes, the injection volume was 25 mL and the retention time was 7 minutes.

Nitrates and nitrites were separated and quantified on a SAX anion exchange cartridge (Water Associates) with a mobile phase consisting of 2.5 mM phosphate buffer flowing at 1 mL per minute. The detector was set at 229 nm, injection volumes were either 25 uL or 200 uL, and the run time was 15 minutes. Prior to HPLC analysis, all samples were filtered through 0.45 um Nylon 66 filters (Rainin Instrument Co., Woburn, MA).

Total Organic Carbon (TOC) Analysis

TOC was determined on a Tocamaster model 915-B (Beckman Instruments, Fullerton, CA) with Matheson Ultra Zero air as carrier gas, flowing at 300 mL per minute. Each sample, 20 uL in volume, was injected with a Hamilton CR-200 200 uL constant rate syringe. Prior to analysis, samples were centrifuged at 12,000 rpm for 20 minutes and then filtered through a Nylon 66 0.45 um filter (Rainin Instrument Co.).

Mass Spectrometry (MS)

MS analyses were performed on a Finnigan 4000 Mass Spectrometer operating in either Electron Impact or Chemical Ionization modes. Samples were analyzed by probe.

Ammonia and pH Analyses

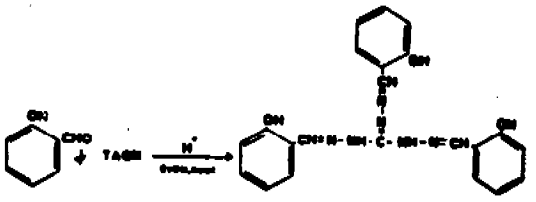
Ammonia and pH were determined with a Corning Model 130 meter (Medford, MA). Ammonia determinations were made with an ion specific electrode model 4209-N30 (A. H. Thomas, Philadelphia, PA) and pH with a Corning pH electrode, model 476022 and Corning calomel reference electrode, model 476002.

Colorimetric Assay - TAGN

Efforts were made to develop a colorimetric assay for TAGN using ninhydrin as color-forming reagent. The reaction mixture consisted of TAGN (1 to 1000 mg/L) and ninhydrin (25 uL to 100 mL of a 0.5 g/100 mL stock solution in distilled, deionized water) in a total volume of 10 mL. Solutions were heated in an 85°C water bath for 10 minutes and then assayed at 285 nm. The method was not used in actual experiments, due to the lack of a linear response over a reasonable range of concentrations of TAGN.

Gas Chromatography (GC) - Derivative Formation

Since TAGN cannot be directly volatilized and analyzed by GC, the formation of derivatives with salicylaldehyde was investigated as a method to permit quantitation. The reaction mixture contained the aqueous sample, 0.1 mL, 0.9 mL absolute ethanol, 0.1 mL glacial acetic acid, and 0.1 mL salicylaldehyde (2 M stock in ethanol). The solution was mixed, heated to 60°C for 20 minutes, diluted with 2.8 mL of ethanol, and injected onto a Hewlett Packard 9835 GC equipped with a 1.9 m long by 0.19 cm diameter copper column packed with 2% Dexil WHP, 100-120 mesh. The oven was set at 320°C, the injector at 350°C, and the flame ionization detector at 350°C. The run time was 20 minutes, and nitrogen carrier gas flowed at 30 mL per minute. The linear response range was insufficient to permit the use of this method for routine analysis.



Thin-Layer Chromatography (TLC)

TAGN and its potential metabolites were chromatographed in two systems: (1) plastic-backed cellulose plates without fluorescent indicator (Eastman, Rochester, NY) developed in a solvent system of butanol/ethyl acetate/water (4/1/1), and (2) silica gel LK5DF plates (Whatman) developed in a solvent system of methanol/water/glacial acetic acid (20/10/1). The compounds were visualized with either alkaline nitroprusside-potassium ferricyanide (10% potassium ferricyanide, 10% sodium nitroprusside, 10% sodium hydroxide, diluted with 3 volumes of distilled, deionized water and diluted 1 to 1 with acetone prior to spraying)[24] or ninhydrin (0.3 g ninhydrin in n-butanol with 3 mL acetic acid, heated at 90°C to 100°C for 5 minutes). Samples, 50 uL, were chromatographed along with standards. In addition, 100-fold concentrates from continuous culture samples were chromatographed. Samples were concentrated by rotary evaporation at 60°C as both acidified and neutral solutions. The R_f's and color development for standards are given in Table 1. Detection limits for 50 uL samples were 1 ug for TAGN, cyanamide, and cyanoguanidine, and 2 ug for urea and carbohydrazide.

TABLE 1. Thin-Layer Chromatographic Analysis of TAGN and Possible Microbially Produced Intermediates.

Compound	Cellulose Plates (Nitroprusside spray)	Silica Plates (Ninhydrin spray)
TAGN	Violet (0.08)	Yellow/brown (0.56)
Carbohydrazide	Peach (0.10)	—
Guanidine	Orange (0 27)	—
Urea	Red/orange (0.40)	—
Cyanamide	Violet (0.74)	—
Cyanoguanidine	Violet (0.56)	—

Nitrosamine Formation

To assess the potential for formation of nitrosamines from TAGN, 1000 mL of TAGN, 200 mg/L in distilled deionized water, was reacted with 1000 mg/L sodium nitrite. The reaction mixture was stirred, and samples were removed at periodic intervals up to 24 hours. Reactions were also run with acidified medium. Samples were chromatographed on cellulose plates as described above. Spots were visualized with sulfanilic acid x-naphthylamine spray (1% sulphanilic acid in 30% acetic acid) and exposed to UV light, producing a red-violet color.

Alkaline Hydrolysis

Results from the analysis of samples from batch studies indicated a potential instability of TAGN in alkaline solutions. This instability prompted an investigation into the alkaline hydrolysis of TAGN. The reaction mixture consisted of 250 mg TAGN dissolved in 25 mL distilled, deionized water in a 50 mL Erlenmeyer flask with continuous stirring. Sodium hydroxide, 0.2 N, was added dropwise to bring the solution pH up to 9. Aliquots of the reaction mixture were removed hourly and analyzed by TLC on the silica gel plates as described previously. Chromatograms were visualized with the nitroprusside-potassium ferricyanide spray, and standards were co-chromatographed with the reaction samples. The reaction was continued until all TAGN had disappeared, at which time the solution was acidified to pH 4 with 5N HCl and re-chromatographed. The reaction mixture was evaporated to dryness in a rotary evaporator at 60°C. The residue was redissolved in hot absolute ethanol, while the ethanol-insoluble material was dissolved in a minimum volume of water. Both the ethanol and water solutions were co-chromatographed.

In separate studies, alkaline hydrolysis was performed in a system with continuous refluxing and an acid trap was used to collect ammonia gas and hydrazines if formed. Studies were run with 200 mg/L TAGN in 0.1 M sodium phosphate buffer at pH 5.7, 7.0, and 8.0 over 3 days, or 5000 mg/L TAGN refluxed for 10 hours. Ammonia collected in the acid traps was determined on an ion specific electrode as described above. Hydrazines were determined by TLC with a solvent system consisting of absolute ethanol/water/HCl (130/40/30) on plastic backed cellulose plates without fluorescent indicator. Chromatograms were visualized by spraying first with 20% Na_2CO_3, followed by Folin-Ciocalteau solutions, and finally, exposure to ammonia fumes to develop the blue color. After all the TAGN had disappeared from the reaction mixture, acidified and alkaline fractions from the 5000 mg/L TAGN refluxing reaction were dried under nitrogen gas and then extracted with methanol. TLC and MS analyses were performed on the sample extracts and standards.

Continuous Cultures

A series of continuous culture studies were run to evaluate the degradation of TAGN and resorcinol under a variety of environmental conditions.

The first set of continuous systems consisted of aerobic and anaerobic (microaerophilic) fermentors, approximately 400 mL total volume, containing TAGN, 200 mg/L; resorcinol, 50 mg/L; isodecyl pelargonate, 1 mg/L; and Paraplex G-54, 1 mg/L, in nutrient broth at varying concentrations as detailed in the results section. After autoclaving the above solution, the resorcinol was added as a filter sterilized solution. The aerobic system was vigorously stirred and aerated, while the anaerobic system was slowly stirred and unaerated. Both systems were maintained at room temperature. Details on pH, retention times, nitrate concentrations and resorcinol levels are given in the results section.

The second set of continuous cultures were aerobic and anaerobic systems run with TAGN, 200 mg/L, in a series of different media; initially in nutrient-rich broth, and gradually reduced to minimal salts medium. The

changes in media composition and results of analyses are presented in the results section. The system configuration and treatment were as as above, and the basal salts were the same as those described in the batch culture section without nitrogen.

The third set of continuous cultures consisted of aerobic and anaerobic formulations containing the following ingredients per liter: K_2HPO_4, 0.75 g; KH_2PO_4, 1.25 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.01 g; NaCl, 0.01 g; yeast extract, 0.5 g; and TAGN, 0.2 g. The concentration of supplemental carbon in the form of glucose and sucrose was changed during the course of the experiment from a total of 2 g/L to 8 g/L, consisting of equal parts of the two sugars. The media were autoclaved and the TAGN added afterwards as a filter sterilized solution which had been passed through a 0.45 um membrane filter. The systems were monitored for retention time, flow rate, pH, ammonia, nitrate, nitrites, TAGN, intermediates, and total organic carbon (TOC). Samples, 50 uL, were spotted along with standards for TLC analysis. Samples for HPLC and TOC analysis were centrifuged at 8000 rpm for 100 minutes and then passed through a 0.45 um membrane filter.

Batch Studies

A series of batch studies was run to evaluate the degradation of TAGN under a variety of environmental conditions. Media used in these studies included the following: basal salts (as described in the continuous culture section) with and without nitrogen and with and without yeast extract, basal salts without nitrogen and with 0.1 g/L sucrose and 0.1 g/L glucose, filtered lake water, trace salts in lake water (per liter: $MgSO_4 \cdot 7H_2O$, 0.05 g; $FeCl_3 \cdot 6H_2O$, 0.05 g; $MnSO_4 \cdot H_2O$, 0.003 g; $Na_2MoO_4 \cdot 2H_2O$, 0.005 g; $ZnSO_4 \cdot 7H_2O$, 0.0006 g; $CuSO_4 \cdot 5H_2O$, 0.0005 g; $CaCl_2 \cdot 6H_2O$, 0.00048 g, KH_2PO_4, 0.34 g; biotin, 0.001 g with and without sucrose, 3g/L; and glucose, 3g/L), nutrient broth, yeast extract, and distilled water. Sterile controls, both autoclaved and filter sterilized through a 0.45 um membrane filter, were investigated. Aerobic (generally 50 mL or 100 mL of media in a 250 mL Delong flask at 30°C on a rotary incubator at 200 rpm) and anaerobic incubations (200 mL of media in a 250 mL screw-topped Erlenmeyer flask at 37°C without shaking) were studied for periods up to three weeks. Flask conditions were usually replicated at least twice. Some incubations included 0.025% sodium sulfide for anaerobiasis.

Batch and continuous cultures were inoculated with organisms from activated sludge (Marlborough Easterly Sewage Treatment Plant, Marlborough, MA) anaerobic sludge digest (Nut Island Sewage Treatment Plant, Boston, MA) and garden soil. One mL samples of the two sludges were combined with 1 g of soil, diluted with 50 mL of lake water, mixed, gravity-filtered through filter paper, and 100 uL used as inoculum in batch cultures and 1 mL in continuous cultures. This inoculum contained between 2.3×10^3 and 3.5×10^3 CFU/mL when grown under aerobic conditions.

Solubility of Paraplex G-54 and Isodecyl Pelargonate

Solutions of isodecyl pelargonate and Paraplex G-54 were prepared in distilled deionized water in acid washed glassware. Solutions were stirred overnight with and without heating. The solutions were analyzed for TOC as described above.

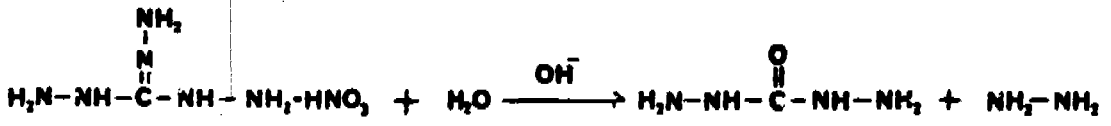
RESULTS

Solubility of Paraplex G-54 and Isodecyl Pelargonate

Both Paraplex G-54 and isodecyl pelargonate were soluble in water at less than 2 mg/L.

Alkaline Hydrolysis

TAGN was rapidly hydrolyzed under alkaline conditions and the reaction rate was greatly accelerated by heating. No evidence was found for ammonia or hydrazine as products of the reaction, but carbohydrazide was detected by TLC and MS analyses. Hydrazine would be expected to be present as a product



of this hydrolysis, but was not detected. The absence of hydrazine may indicate secondary reactions have taken place which resulted in the formation of other unidentified products. MS chromatograms for TAGN produced by EI and CI probe analysis are illustrated in Figs. 2 and 3. The parent ion (104 m/z for EI and 105 m/z for CI) is present. The most prominent ion is the 58 m/z. See Table 2 for fragmentation patterns. The MS chromatogram for carbohydrazide is shown for EI probe analysis in Fig. 4. The parent ion, 90 m/z, is present and m/z 31 is the most prominent ion (Table 2).

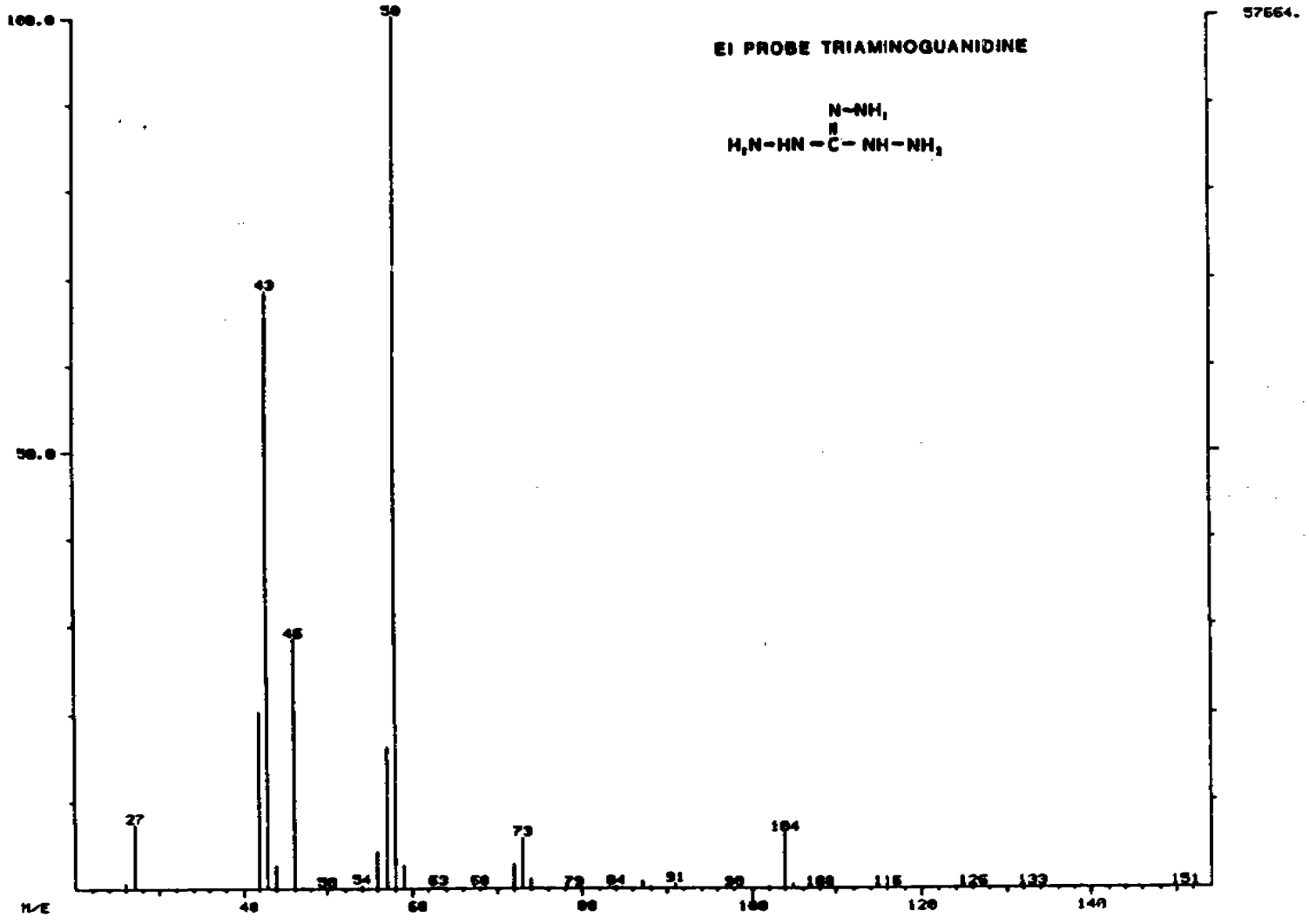


Figure 2. EI mass spectrum of TAGN.

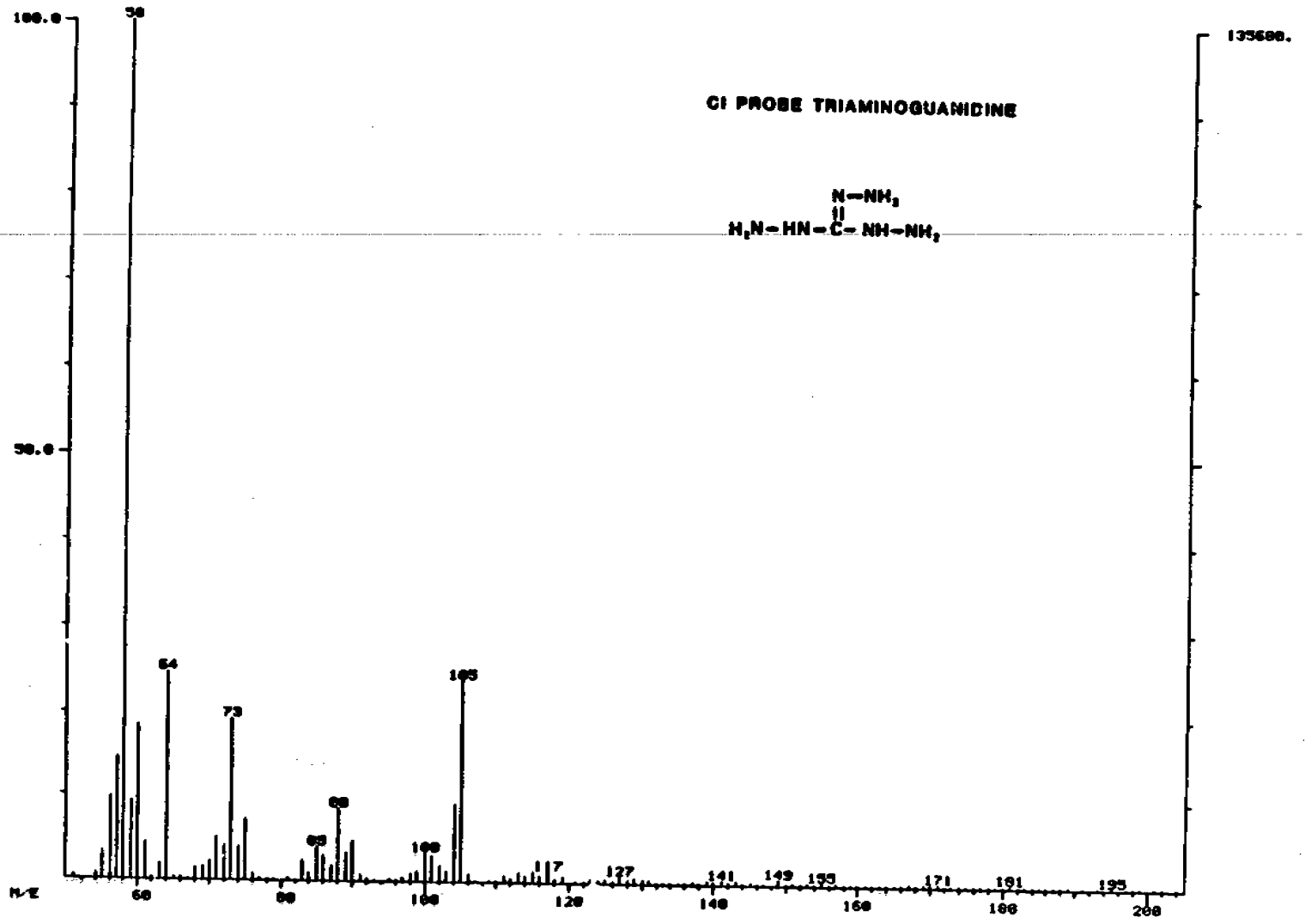


Figure 3. CI mass spectrum of TAGN.

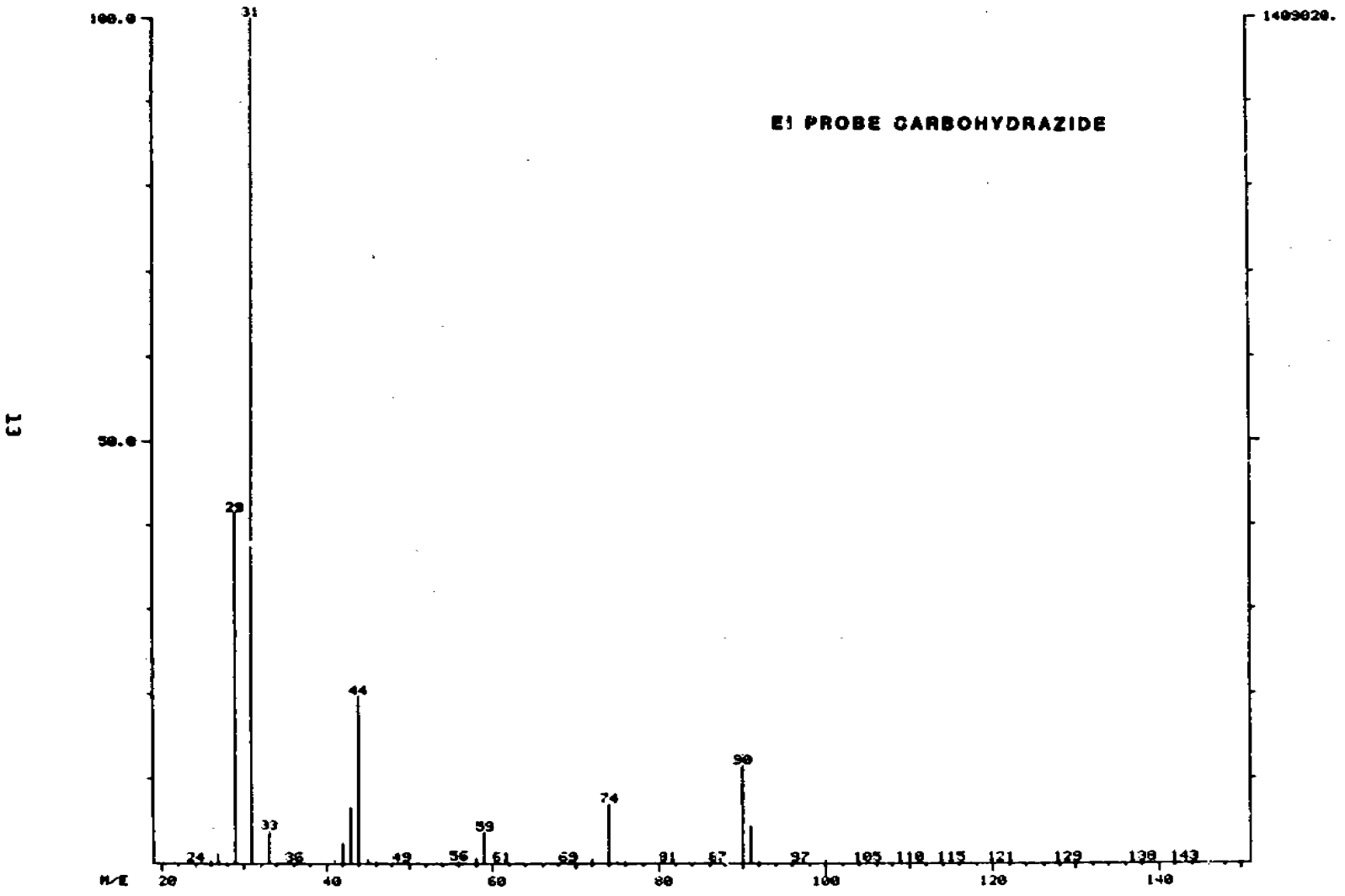
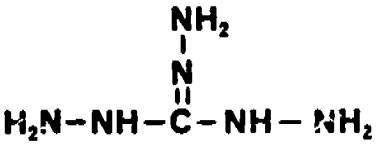
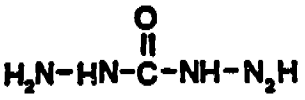
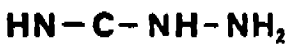
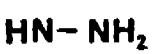
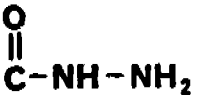
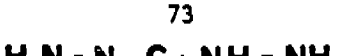
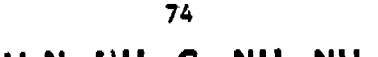


Figure 4. EI mass spectrum of carbohydrazide.

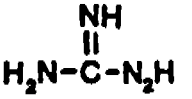
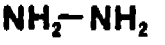
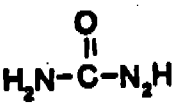
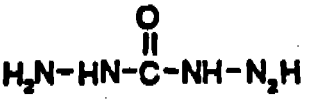
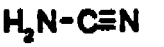
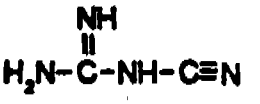
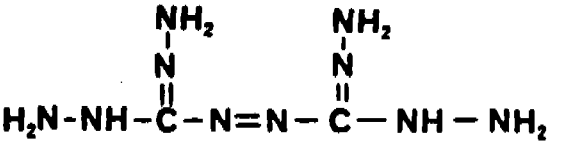
TABLE 2. Molecular Ion Fragments Detected from MS Analysis by EI Probe.

Molecular ions m/z	Compound	
	Triaminoguanidine	Carbohydrazide
Parent ion	104 	90 
Most prominent ion	58 	31 
Other fragments	43	59 
	73 	74 

Intermediates

The intermediates which would most likely be produced during the microbial biotransformation of TAGN are illustrated in Table 3. These compounds were monitored in batch and continuous culture effluent samples by HPLC or TLC.

TABLE 3. Possible Intermediates Formed from TAGN

Compound	Structure
1. Guanidine	
2. Hydrazine	
3. Urea	
4. Carbohydrazide (1,3-diaminourea)	
5. Cyanamide	
6. Cyanoguanidine	
7. Other	

Continuous Cultures

The changes in media composition and results of analysis of retention times and pH in the first set of continuous cultures under aerobic and anaerobic conditions receiving 200 mg/L TAGN, 50 mg/L resorcinol, and traces of isodecyl pelargonate and Paraplex G-54 are presented in Table 4. The

TABLE 4. Results of Analysis and Changes in Media Composition for The First Set of Continuous Cultures.

System	Days	Retention time (days) $\bar{X} \pm$ 1S.D.	pH effluent $\bar{X} \pm$ 1S.D.	Medium[a] Composition(g/L)
Aerobic	1-35	4.06±0.70(9)[b]	8.28±0.72(5)	2.000
	36-109	4.35±1.05(35)	8.49±0.16(9)	1.000
	110-147	4.01±0.84(18)	8.04±0.15(5)	0.250
	148-170	4.44±0.95(11)	8.40±0.10(3)	0.500
	171-183	4.44±0.26(5)	8.50±0.14(2)	1.000
Anaerobic	1-27	3.94±0.09(5)	7.43±0.17(4)	1.000
	28-62	4.40±1.27(2)	7.56±0.29(5)	0.500
	63-106	4.65±0.65(11)	7.14±0.18(5)	0.250
	107-125	6.56±0.99(5)	7.10±0.00(2)	0.125
	126-157	6.47±0.99(6)	7.87±0.12(3)	0.063

[a]Changes in concentration of nutrient broth, all systems received TAGN, 200 mg/L; resorcinol, 50 mg/L; isodecyl pelargonate, 1 mg/L, and Paraplex G-54, 1 mg/L.

[b]Numbers in parentheses represent samples evaluated during time frame (days).

disappearance of nitrates and resorcinol is illustrated in Fig. 5 for the anerobic system. Resorcinol was also completely degraded in the aerobic system, but the nitrates passed through the system unchanged. HPLC analysis of the effluents revealed no other significant UV-absorbing peaks, which would be indicative of incomplete degradation. Nitrates, which arise from the dissociation of TAGN in solution, are reduced during denitrification in the anerobic system. No significant nitrite levels were detected during the same analysis on the exchange column. As the nutrient broth load in the system was reduced, the point was reached where insufficient supplemental carbon was present to provide the needed electron donors to reduce all the nitrate present. This incomplete reduction of nitrate is reflected in Fig. 5 at around day 110, after the concentration of nutrient broth had been reduced to 0.125 g/L.

The results from the second set of continuous cultures demonstrated the requirement for supplemental carbon for TAGN degradation under both aerobic and anaerobic conditions. TLC analysis of continuous culture samples revealed that TAGN was completely degraded, without evidence for intermediates, provided sufficient alternative carbon was provided. Details on media composition, pH, retention times, nitrates and TAGN are presented in Table 5.

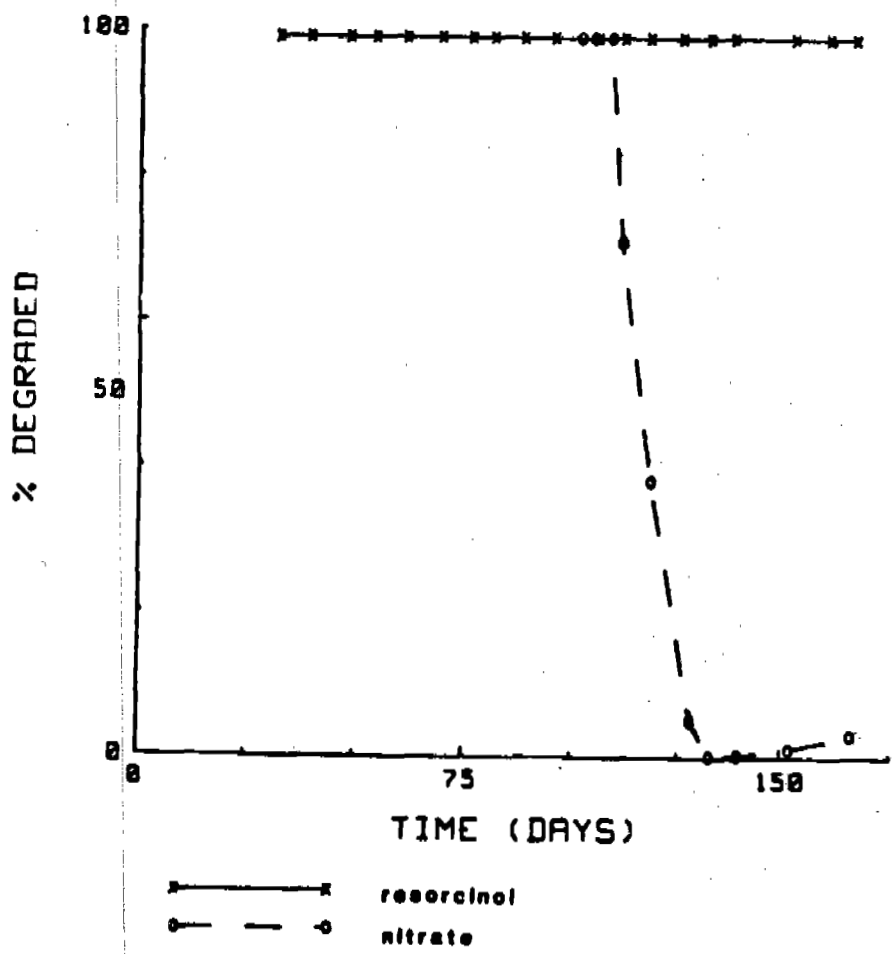


Figure 5. Degradation of nitrates and resorcinol in the anaerobic continuous flow system.

TABLE 5. Results of Analysis and Changes in Media Composition for the Second Set of Continuous Cultures

System	Days	Retention Time, days $\bar{X}\pm1$ S.D.	pH effluent $\bar{X}\pm1$ S.D.	Media[a] Composition	Nitrate Removal (%)	TAGN Disappearance
Aerobic	1-11	3.50±0.10(3)[b]	8.65±0.21(2)	Nutrient broth (4 g/L)	-	Complete
	12-20	7.70±0.42(2)	-	Nutrient broth (1 g/L)	0(1)	Complete
	21-28	4.70±0.69(5)	8.35±0.49(2)	Salts[c] + glucose (1 g/L)	13(1)	Complete
	29-41	3.32±0.56(5)	7.20±0.14(2)	Salts	47(1)	None
	42-49	4.22±0.52(6)	-	Nutrient broth (8 g/L)	0(1)	Complete
Anaerobic	1-11	4.69±1.26(4)	7.50±0.14(2)	Nutrient broth (4 g/L)	-	Complete
	12-26	4.36±0.56(5)	7.75±0.12(2)	Nutrient broth (1 g/L)	9(1)	Partial
	27-32	4.02±0.46(4)	7.10±0.00(1)	Salts + glucose (1 g/L)	93(1)	Partial
	33-41	3.90±1.63(4)	7.10±0.00(1)	Salts		-
	42-48	3.55±0.17(4)	7.80±0.00(1)	Nutrient broth (8 g/L)	-	-

[a]All systems received 200 mg/L TAGN

[b]Numbers in parentheses represent samples evaluated during the time frame.

[c]Salts consisted of the following per liter: K_2HPO_4, 1.25 g; KH_2PO_4, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; $CaCl_2$, 0.01 g; and NaCl, 0.01 g.

In the third set of continuous cultures (Table 6), complete disappearance of TAGN was achieved in both the aerobic and anaerobic continuous flow systems with glucose and sucrose present (at least 6g/L) as supplemental carbon. As before, sufficient alternate carbon was required for complete disappearance of TAGN. Also as before, no intermediates were detected in effluent samples, indicating complete degradation. No significant buildup of concentrations of nitrates, nitrites, or ammonia was detected. The nitrate concentrations decreased in the effluent as compared to influent levels, and ammonia levels remained low. Complete disappearance of TAGN was achieved when the concentration of the sugars was at least 6 g/L under aerobic conditions, and 8 g/L under anaerobic conditions. TOC analysis indicated reductions of 68% to 97% up to day 44. The pH of the influents to both systems remained between 5.0 and 6.0 throughout the study.

Batch Culture Studies

The results from the series of batch culture studies supported the results found in the continuous cultures. TAGN was degraded in systems where sufficient supplemental carbon was provided under either aerobic or anaerobic conditions. In incubations where TAGN had disappeared, no evidence was found for metabolic intermediates, despite concentrating some solutions for better detection. Ammonia concentrations remained in the low ppm (< 3). In general, complete disappearance of TAGN occurred in 6 days to two weeks from the start of the incubations with the inoculum described in the materials and methods section. Evaluations of sterile controls indicated that TAGN was stable under most conditions provided the medium was maintained at a pH below 7.

Nitrates, produced from TAGN in solution, are reduced to nitrites and eventually nitrogen gas under anerobic or microaerophilic conditions, provided sufficient carbon is present. Nitrate concentrations decreased in the active incubation with 1 g/L yeast extract, while the concentration of nitrate remained unchanged in the sterile controls and in the flask with a lower concentration of yeast extract.

The results from these studies clearly demonstrate the need for supplemental carbon, not only for the complete degradation of TAGN, but also for the complete reduction of the nitrate that arises from TAGN.

TABLE 6. Results of Analysis and Changes in Media Composition for the Third Set of Continuous Cultures.

System	Days	Retention Time (Days) $\bar{X}\pm1$ S.D.	pH effluent $\bar{X}\pm1$ S.D.	Media[a] Composition (g/L sugar)	Ammonia (mg/L) in effluent $\bar{X}\pm1$ S.D.	Nitrates (mg/L) in effluent $\bar{X}\pm1$ S.D.	TAGN disappearance	TOC removal (%)
Aerobic	0-5	5.3±0.5	---	2	3.0±0.2	0.8±0.7	None	---
	6-12	5.5±0.9	---	2	11.5±3.3	4.9±1.0	None	87.4±0.6
	13-21	4.6±0.4	---	4	7.7±0.6	3.3±1.6	Partial	88.4±0.4
	22-35	5.7±0.6	---	8	24.8±4.6	6.1±1.8	Complete	95.5±0.0
	36-44	5.5±0.4	---	8	29.4±2.5	4.1±0.0	Complete	96.7±0.0
	45-59	---	5.7±0.1	8	6.3±1.3	---	Complete	---
	60-63	4.2±0.0	5.8±0.0	6	3.3±0.0	---	Complete	---
	64-70	6.5±1.4	6.0±0.1	6	6.9±6.5	0.2±0.0	Complete	---
	71-80	11.0±5.6	6.2±0.2	6	15.5±9.9	1.6±2.2	Complete	---
	81-94	3.8±1.2	5.6±0.2	5	0.7±0.6	0.5±0.1	Partial	---
Anaerobic	0-5	6.3±0.8	---	2	0.9±0.3	---	None	---
	6-12	5.5±0.8	---	2	3.5±0.1	---	None	86.8±3.1
	13-21	5.5±1.3	---	4	0.9±0.8	---	Partial	87.5±0.6
	22-35	6.7±1.1	---	8	42.3±6.8	---	Complete	68.3±0.0
	36-44	5.4±0.5	---	8	33.4±2.2	---	Complete	75.1±0.0
	45-59	7.9±6.4	3.1±0.0	8	33.8±6.8	---	Complete	---
	60-63	6.7±0.0	3.1±0.0	8	0.3±0.0	---	Partial	---
	64-70	6.4±1.3	3.2±0.1	6	0.3±0.2	---	Partial	---
	71-80	3.5±0.4	3.1±0.1	7	0.7±0.2	5.4±3.6	Partial	---
	81-94	3.4±0.5	2.9±0.1	8	0.2±0.2	5.3±7.2	Partial	---

[a] All media contain equal parts glucose and sucrose.

DISCUSSION

The results from the biodegradation studies with TAGN have demonstrated that this compound is amenable to biological treatment, provided the proper environmental conditions are present. Sufficient supplemental carbon must be provided in the incubation medium to offset the nitrogen-rich TAGN and provide the needed carbon for energy. This process, known as cometabolism, implies that the metabolism of TAGN is, in fact, a secondary, nonspecific, activity of the enzymes produced during the metabolism of the energy-rich supplemental carbon provided. This activity occurs under either aerobic or anaerobic conditions, however, since nitrates are formed upon dissociation of TAGN in solution. These anions are only reduced to nitrogen gas under anaerobic or microaerophilic conditions with sufficient alternate carbon present as well.

No evidence was found for the production of intermediates from TAGN, with the exception of carbohydrazide under alkaline conditions. The absence of these compounds (urea, guanidine, cyanamide, cyanoguanidine, hydrazine) implies complete degradation of TAGN by microorganisms. Satriana[9] noted that triaminoguanidine, as a free base, decomposed rapidly in moist air and in aqueous media, and diaminourea (carbohydrazide) was identified as the decomposition product.

A problem with the above work was the absence of a quantitative method for TAGN. The HPLC used for nitrate/nitrite analysis was originally developed to follow the concentration of TAGN. However, since it was found that TAGN dissociates in solution, only the nitrate portion of the compound is "seen" by the LC detector. TLC permitted sensitive qualitative detection of TAGN.

The importance of supplemental carbon in providing the electron donors or energy to permit TAGN to be cometabolized has been noted previously with a number of munitions compounds, including nitroguanidine, RDX, and HMX. In general, with nitrogen-rich compounds such as these, the C/N ratio of the compounds themselves is so low that alternate carbon energy is necessary for metabolism.

CONCLUSIONS

Triaminoguanidine nitrate was shown to be biodegradable under both aerobic and anaerobic conditions. A requirement for supplemental carbon was identified, both for the cometabolism of the organic-nitrogen portion of the propellant, as well as for the reduction of the dissociated nitrates. The nitrate reduction (denitrification) will not occur under aerobic conditions; thus, an anaerobic biological system may be the most beneficial in treating nitramine laden wastewaters. RDX and HMX have previously been shown to degrade anaerobically and not aerobically, and would therefore benefit from this anaerobic system as well.

Triaminoguanidine nitrate decomposed to carbohydrazide under alkaline conditions. No significant levels of potential intermediates were identified in the biological systems. Resorcinol was also shown to be readily biodegraded, as expected from reports in the literature. Isodecyl pelargonate and Paraplex G-54 were only sparingly soluble. Reports in the literature indicate that this class of compound would be biodegradable.

This document reports research undertaken at the US Army Natick Research and Development Command and has been assigned No. NATICK/TR-95/045 in the series of reports approved for publication.

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